Data Evaluation Report on the Acute Toxicity of 4775453 (M07; Metabolite of BAS 800 H) to Algae (*Pseudokirchneriella subcapitata*)

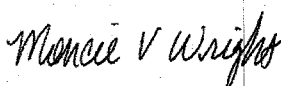PMRA Submission Number: 2008-0431
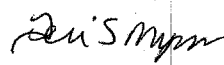PMRA Document ID: 1664711   EPA MRID Number: 47560301

Data Requirement:

	PMRA DATA CODE	9.8.2
	EPA DP Barcode	349851
	OECD Data Point	IIA 8.4
	EPA MRID	47560301
	EPA Guideline	OPPTS 850.5400 (123-2)

Test material: 4775453 (M07; metabolite of BAS 800 H)   **Purity:** 95.4%
Common name: Saflufenacil metabolite
Chemical name: IUPAC: N-{4-chloro-2-fluoro-5-[({[isopropyl(methyl)amino]sulfonyl}amino)carbonyl]phenyl}-N'-methylurea
CAS name: Not reported
CAS No.: Not reported
Synonyms: Not reported

Primary Reviewer: Moncie Wright   **Signature:**
Staff Scientist, Cambridge Environmental   **Date:** 11/25/08

Secondary Reviewer: Teri S. Myers   **Signature:**
Senior Scientist, Cambridge Environmental   **Date:** 12/01/08

Primary Reviewer: Anita Pease   **Date:** 06/09/09
Senior Biologist, U.S. EPA

Secondary Reviewer: Ann Lee   **Date:** 06/09/09
HC-PMRA-EAD

Secondary Reviewer: Farzad Jahromi   **Date:** 06/09/09
DEWHA-APVMA

Company Code	BAZ
Active Code	SFF
Use Site Category:	13 (terrestrial feed crops) and 14 (terrestrial food crops)
EPA PC Code	118203

CITATION: Porch, J.R., Kendall, T.Z., Krueger, H.O., and Holmes, C.M. 2008. BAS 800 H Metabolite M07: A 96-hour Toxicity Test with the Freshwater Alga (*Pseudokirchneriella subcapitata*). Unpublished study performed by Wildlife International, Ltd., Easton, Maryland. Wildlife International Study No.: 147A-242. Study sponsored by BASF Corporation, Research Triangle Park, North Carolina. BASF Study No.: 355548. Study completed August 28, 2008.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to aquatic nonvascular plants. It is not intended to prescribe conditions to any external party for conducting this study nor to establish

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absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of the freshwater green algae (*Pseudokirchneriella subcapitata*) were exposed to 4775453 (M07; metabolite of BAS 800 H) at nominal concentrations of 0 (negative control), 3.9, 6.5, 11, 18, and 30 mg a.i./L under static conditions. Mean-measured concentrations were <2.10 (<LOQ, control), 3.8, 6.4, 11, 18, and 29 mg a.i./L.

No endpoint was sensitive to treatment with the Saflufenacil metabolite 4775453, with NOAEC and EC_{50} values of 29 and >29 mg a.i./L, respectively.

There were no signs of adherence to test chambers or aggregation/flocculation of algae in the controls or treatment groups, and there were no noticeable changes in cell morphology at study termination.

This toxicity study is classified as **ACCEPTABLE** to the **U.S. EPA** and as **FULLY RELIABLE** to **PMRA and APVMA** as it is scientifically sound and satisfies the guideline requirement for a nonvascular aquatic plant toxicity study with the freshwater green algae, *Pseudokirchneriella subcapitata*.

Results Synopsis

Test Organism: *Pseudokirchneriella subcapitata*
Test Type (Flow-through, Static, Static Renewal): Static

Cell density
EC_{05}:   >29 mg a.i./L   95% C.I.: N/A
EC_{50}:   >29 mg a.i./L   95% C.I.: N/A
NOAEC: 29 mg a.i./L
Probit Slope: N/A

Biomass (Area Under the Growth Curve)
EC_{05}:   >29 mg a.i./L   95% C.I.: Not calculable
EC_{50}:   >29 mg a.i./L   95% C.I.: N/A
NOAEC: 29 mg a.i./L
Probit Slope: N/A

Growth Rate
EC_{05}:   >29 mg a.i./L   95% C.I.: N/A
EC_{50}:   >29 mg a.i./L   95% C.I.: N/A
NOAEC: 29 mg a.i./L

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Probit Slope: N/A

Yield
EC_{05}:   >29 mg a.i./L   95% C.I.: N/A
EC_{50}:   >29 mg a.i./L   95% C.I.: N/A
NOAEC: 29 mg a.i./L
Probit Slope: N/A

Endpoint(s) Effected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:	This study was conducted following OECD Guidelines for the Testing of Chemicals 201: *Freshwater Alga and Cyanobacteria, Growth Inhibition Test*, Official Journal of the European Communities, No. L383, Method C.3: *Algal Inhibition Test*, and USEPA Series 850 – Ecological Effects Test Guidelines (*draft*), OPPTS No. 850.5400, *Algal Toxicity, Tiers I and II*. No deviations from OPPTS 850.5400 were noted.
COMPLIANCE:	Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Certification statements were provided. This study was conducted in compliance with U.S. EPA FIFRA GLP standards (40 CFR Part 160 and 792; 1989), OECD Principles of GLP (ENV/MC/CHEM (98) 17) and JMAFF (1999), with the following exception: Periodic analyses of well water for potential contaminants were not performed according to GLP standards, but were performed using a certified laboratory and standard U.S. EPA analytical methods.
A. MATERIALS:	
1. Test material	4775453 (metabolite of BAS 800 H)
Description:	Solid
Lot No./Batch No. :	L67-196 (Batch No.)
Purity:	95.4%
Stability of compound under test conditions:	The 96-hour measured concentrations yielded recoveries of *ca.* 93 to 97% of nominal test concentrations, indicating that 4775453 was stable under test

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conditions.
(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

**Storage conditions of
test chemicals:**   Stored under ambient conditions.

Physicochemical properties of 4775453.

Parameter	Values	Comments
Water solubility at 20°C	Not reported.	
Vapor pressure	Not reported.	
UV absorption	Not reported.	
pKa	Not reported.	
Kow	Not reported.	

2. Test organism:

Name: Freshwater green algae, *Pseudokirchneriella subcapitata*
EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. capricorntum, and a freshwater diatom is tested.

OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported

Strain: Not reported
Source: In-house cultures originally obtained from the University of Toronto Culture
                                     Collection
Age of inoculum: 2 weeks
Method of cultivation: Grown in freshwater algal medium.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study   A range-finding study was conducted with a negative control, and nominal test concentrations of 0.49, 1.6, 5.4, 18, and 60 mg/L. After 96 hours, cell density in the negative control was 1.93×10^6 cells/mL, yielding inhibitions of 3.1, 3.7, 5.0, -8.1, and 1.1%.

b. Definitive Study

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Table 1: Experimental Parameters

Parameter	Details	Remarks
		Criteria
Acclimation period: Culturing media and conditions: (same as test or not) Health: (any mortality observed)	Continuous Freshwater algal medium Same as test. Not reported.	*EPA recommends two week acclimation period.* *OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.*
Test system Static/static renewal Renewal rate for static renewal	Static N/A	*EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).*
Incubation facility	Test vessels were placed on an orbital shaker table in an environmental chamber.	
Duration of the test	96 hours	*EPA requires: 96-120 hours* *OECD: 72 hours*
Test vessel Material: *(glass/stainless steel)*	Glass	

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Parameter	Details	Remarks
		Criteria
Size: Fill volume:	250 mL 100 mL	*OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*
Details of growth medium name pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (**for marine algae**):	 7.0-7.5 8.0-8.1 Yes. $NaHCO_3$. N/A	*OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.* *EPA recommends 20X-AAP and chelating agents (e.g. EDTA) in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91 and D 3978-80 (reapproved 1987).*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Standard nutrient medium was used.	

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Parameter	Details	Remarks
		Criteria
Dilution water source/type: pH: salinity (**for marine algae**): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Purified well water. Adjusted to 7.5. N/A Filter sterilized. Not reported. Not reported. See Reviewer's Comments. None Detected. Not reported.	*EPA pH: <u>Skeletonema costatum</u>= ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.* *OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.*
Indicate how the test material is added to the medium (added directly or used stock solution)	A stock solution was prepared at the highest test concentration, which was then serially diluted.	
Aeration or agitation	Agitation, 100 rpm	
Initial cells density	1.0×10^4 cells/mL	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Anabaena flos-aquae, cell counts on day 2 are not required.* *OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for <u>S. capricornutum</u> and <u>S. subspicatus</u>. When other species are used the biomass should be comparable.*

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Parameter	Details	Remarks
		Criteria
Number of replicates Control: Solvent control: Treatments:	3 N/A 3	*EPA requires a negative and/or solvent control with 3 or more replicates per doses. <u>Navicula</u> sp.tests should be conducted with four replicate.* *OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test.*
Test concentrations Nominal: Mean-Measured:	0 (negative control), 3.9, 6.5, 11, 18, and 30 mg a.i./L <2.10 (<LOQ, control), 3.8, 6.4, 11, 18, and 29 mg a.i./L	*EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.*
Solvent (type, percentage, if used)	N/A	

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Parameter	Details	Remarks
		Criteria
Method and interval of analytical verification LOQ: LOD:	Test samples collected at 0 and 96 hours, matrix fortification standards, and calibration standards were analyzed using HPLC with UV (220 nm) detection. 2.10 mg a.i./L 0.0167 mg a.i./L	
Test conditions Temperature: Photoperiod: Light intensity and quality:	23.9 – 24.6°C Continuous 4120-4710 lux Cool white fluorescent lighting	*EPA temperature: Skeletonema: 20EC, Others: 24-25EC; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)* *OECD recommended the temperature in the range of 21 to25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.*
Reference chemical (if used) name: concentrations:	N/A	
Other parameters, if any	None.	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks
		Criteria
Parameters measured including the	-cell density	

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Parameters	Details	Remarks
		Criteria
growth inhibition/other toxicity symptoms	- biomass (area under the growth curve) -growth rate - yield	*EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*
Measurement technique for cell density and other end points	Cell counts were conducted daily using an electronic particle counter. Area under the curve, yield, and growth rate were described as being calculated based on cell density data.	*EPA recommends the measurement technique of cell counts or chlorophyll a* *OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).*
Observation intervals	Every 24 hours.	*EPA and OECD: every 24 hours.*
Other observations, if any	N/A	
Indicate whether there was an exponential growth in the control	Yes, cell density in the negative control was 200×10^4 cells/mL at test termination.	*EPA requires control cell count at termination to be 2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Were raw data included?	Yes.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

After 96 hours of exposure, cell density averaged 200×10^4 cells/mL in the negative control, yielding inhibitions of -2, -2, -4, -4, and -1% as compared to the negative control in the mean-measured 3.8, 6.4, 11, 18, and 29 mg a.i./L treatment groups, respectively. The NOAEC and EC_{50} values based on cell density were 29 and >29 mg a.i./L,

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respectively.

Biomass (area under the growth curve) averaged 824 x 10^5 cells/ mL*hour in the negative control, yielding inhibitions of 2, 6, 4, 8, and 4% as compared to the negative control. The NOAEC and EC_{50} values based on biomass were 29 and >29 mg a.i./L, respectively.

Growth rate averaged 0.055 cells/mL/hour in the negative control, yielding inhibitions of 0, 0, -1, -1, and 0% as compared to the negative control. The NOAEC and EC_{50} values based on growth rate were 29 and >29 mg a.i./L, respectively.

Yield averaged 199 x 10^4 cells/mL in the negative control, yielding inhibitions of -2, -2, -4, -4, and -1% as compared to the negative control. The NOAEC and EC_{50} values based on yield were 29 and >29 mg a.i./L, respectively.

The study authors reported that there were no signs of adherence to test chambers or aggregation/flocculation of algae in the controls or treatment groups, and there were no noticeable changes in cell morphology.

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Table 3: Effect of 4775453 on algal growth (*Pseudokirchneriella subcapitata*)

Mean-Measured and (Nominal) Concentrations (mg a.i./L)	Initial Cell density (x 10⁴ cells/mL)	Cell density (x 10^4 cells/mL) at				
		24 hours	48 hours	72 hours	96 hours	
					Cell count	% inhibition
Negative control	1.0	7.09	39.23	200.49	200.01	N/A
3.8 (3.9)	1.0	6.78	38.44	193.24	204.15	-2
6.4 (6.5)	1.0	6.87	36.96	178.54	203.59	-2
11 (11)	1.0	6.8	35.72	186.31	208.23	-4
18 (18)	1.0	6.86	34.81	173.58	208.66	-4
29 (30)	1.0	6.92	36.86	187.22	201.03	-1

N/A- Not Applicable

Table 4: Effect of 4775453 on algal growth (*Pseudokirchneriella subcapitata*)

Mean-Measured and (Nominal) Concentrations (mg a.i./L)	Initial Cell Density (x10⁴ cells/mL)	Mean Growth Rate (cells/mL/hour)		Mean Area Under the Growth Curve (Biomass) (x10⁵ cells/mL*hour)	
		0-96 Hours	Percent Inhibition	0-96 hours	Percent Inhibition
Negative control	1.0	0.0552	N/A	823.9	N/A
3.8 (3.9)	1.0	0.0554	0	808.9	2
6.4 (6.5)	1.0	0.0554	0	769.6	6
11 (11)	1.0	0.0556	-1	790.7	4
18 (18)	1.0	0.0556	-1	758.6	8
29 (30)	1.0	0.0552	0	787.2	4

N/A- Not Applicable

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Table 5: Statistical endpoint values.

Statistical Endpoint	Cell density	Biomass (Area under the Growth Curve)	Yield	Growth Rate
NOAEC or EC$_{05}$ (mg a.i./L)	29	29	29	29
LOAEC	ND	ND	ND	ND
IC$_{50}$ or EC$_{50}$ (mg a.i./L) (95% C.I.)	>29	>29	>29	>29
Other (EC$_{10}$ and EC$_{90}$)	ND	ND	ND	ND
Reference chemical, if used IC$_{50}$/EC$_{50}$	N/A	N/A	N/A	N/A

ND – not determined

B. REPORTED STATISTICS:

Statistical analysis was performed for the endpoints of cell density, biomass (area under the growth curve), yield, and growth rate. The data was tested for normality and for homogeneity of variance. An ANOVA and Dunnett's test were used to compare the treatment groups to the control. The ECx values and their confidence intervals were determined using nonlinear regression analysis. The NOAEC was determined based on an evaluation of the concentration-response pattern and the results of the statistical analyses. All statistics were conducted using SAS software.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Replicate data for all endpoints were tested for normality and homogeneity. If the assumptions of ANOVA were met, the NOAEC value was determined using the parametric Dunnett's and William's Test. If the assumptions were not met, the NOAEC value was determined using the non-parametric Steels or Kruskal-Wallis Test. All NOAEC values were determined using Toxstat Statistical Software. ECx values (with 95% C.I.) and probit slopes were determined using probit analyses via Nuthatch Statistical Software. All toxicity values were determined using the 96-hour mean-measured concentrations. Growth rate values were multiplied by 10,000 before entry into Toxstat.

Cell density
EC$_{05}$:   >29 mg a.i./L   95% C.I.: N/A
EC$_{50}$:   >29 mg a.i./L   95% C.I.: N/A
NOAEC: 29 mg a.i./L
Probit Slope: N/A

Biomass (Area Under the Growth Curve)
EC$_{05}$:   >29 mg a.i./L   95% C.I.: Not calculable

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EC_{50}:   >29 mg a.i./L   95% C.I.: N/A
NOAEC: 29 mg a.i./L
Probit Slope: N/A

Growth Rate
EC_{05}:   >29 mg a.i./L   95% C.I.: N/A
EC_{50}:   >29 mg a.i./L   95% C.I.: N/A
NOAEC: 29 mg a.i./L
Probit Slope: N/A

Yield
EC_{05}:   >29 mg a.i./L   95% C.I.: N/A
EC_{50}:   >29 mg a.i./L   95% C.I.: N/A
NOAEC: 29 mg a.i./L
Probit Slope: N/A

D. STUDY DEFICIENCIES:

There were no study deficiencies.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions agreed with the study author's. There were no apparent effects of treatment on algal growth.

Results from the periodic screening analysis of the dilution water indicated the presence of the following components: calcium (38.7 mg/L), chloride (4.2 mg/L), fluoride (0.55 mg/L), magnesium (14.6 mg/L), potassium (6.97 mg/L), sodium (19.8 mg/L) and sulfate (6.0 mg/L).

All test solutions appeared clear and colorless.

All validity criteria were met. Cell density increased in the negative control by a factor of at least 16X in 72 hours. The coefficient of variation of average specific growth rates during the whole test period (0-96 hour) in replicate controls was 0.37%, which did not exceed 7%. The mean coefficient of variation for section-by-section growth rates over the entire test period (days 0-1, 1-2, 2-3, 3-4) in the controls was 67.6%, which exceeds 35%. However the mean coefficient of variation for section-by-section growth rates over the first 72 hours of the test (days 0-1, 1-2, and 2-3) in the controls was 10.6%. Therefore, the test was considered valid by the study authors.

The in-life portion of the definitive algal toxicity test was conducted between July 24 and 28, 2008.

F. CONCLUSIONS:

This study is scientifically sound and is classified as ACCEPTABLE to the U.S. EPA and as FULLY RELIABLE to PMRA and APVMA. No endpoint was sensitive to treatment with the Saflufenacil metabolite, with NOAEC and EC_{50} values of 29 and >29 mg a.i./L, respectively.

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Test Organism: *Pseudokirchneriella subcapitata*
Test Type (Flow-through, Static, Static Renewal): Static

Cell density
EC_{05}: >29 mg a.i./L   95% C.I.: N/A
EC_{50}: >29 mg a.i./L   95% C.I.: N/A
NOAEC: 29 mg a.i./L
Probit Slope: N/A

Biomass (Area Under the Growth Curve)
EC_{05}: >29 mg a.i./L   95% C.I.: Not calculable
EC_{50}: >29 mg a.i./L   95% C.I.: N/A
NOAEC: 29 mg a.i./L
Probit Slope: N/A

Growth Rate
EC_{05}:   >29 mg a.i./L   95% C.I.: N/A
EC_{50}: >29 mg a.i./L   95% C.I.: N/A
NOAEC: 29 mg a.i./L
Probit Slope: N/A

Yield
EC_{05}: >29 mg a.i./L   95% C.I.: N/A
EC_{50}: >29 mg a.i./L   95% C.I.: N/A
NOAEC: 29 mg a.i./L
Probit Slope: N/A

Endpoint(s) Effected: None

III. REFERENCES:

1. Organization for Economic Cooperation and Development. 2006. OECD Guidelines for the Testing of Chemicals. Guideline 201: *Freshwater Alga and Cyanobacteria, Growth Inhibition Test*.
2. Official Journal of the European Communities. 1992. No. L383. Method C.3: *Algal Inhibition Test*.
3. U.S. Environmental Protection Agency. 1996. Series 850 - Ecological Effects Test Guidelines (draft), OPPTS Number 850.5400: *Algal Toxicity, Tiers I and II*.
4. ASTM Standard Guide E 1218-97A. 1997. *Standard Guide for Conducting Static 96-Hour Toxicity Tests with Microalgae*. American Society for Testing and Materials. Philadelphia, PA.
5. Bruce, Robert D. and Donald J. Versteeg. 1992. A Statistical Procedure for Modeling Continuous Toxicity Data. *Environmental Toxicology and Chemistry*. 11: 1485-1494.
6. The SAS System for Windows. 1999. Version 8.2. SAS Institute Inc. Cary, North Carolina.

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

```
4775453 & P subcapitata 96-hr cell density (mg/L)
File: 0301c          Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies
--------------------------------------------------------------------------
```

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.206	4.356	6.876	4.356	1.206
OBSERVED	0	6	6	6	0

```
--------------------------------------------------------------------------
Calculated Chi-Square goodness of fit test statistic =   3.7645
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.




4775453 & P subcapitata 96-hr cell density (mg/L)
File: 0301c          Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
--------------------------------------------------------------------------

D = 67492677097.313

W =    0.962

Critical W (P = 0.05) (n = 18) = 0.897
Critical W (P = 0.01) (n = 18) = 0.858
--------------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.




4775453 & P subcapitata 96-hr cell density (mg/L)
File: 0301c          Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
--------------------------------------------------------------------------

Calculated H statistic (max Var/min Var) =   84.05
Closest, conservative, Table H statistic = 1362.0   (alpha = 0.01)

Used for Table H ==>      R (# groups) =   6,    df (# reps-1) =      2
Actual values    ==>      R (# groups) =   6,    df (# avg reps-1) =  2.00


--------------------------------------------------------------------------

Data PASS homogeneity test. Continue analysis.
```

Data Evaluation Report on the Acute Toxicity of 4775453 (M07; Metabolite of BAS 800 H) to Algae (*Pseudokirchneriella subcapitata*)

PMRA Submission Number: 2008-0431
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```
NOTE: This test requires equal replicate sizes. If they are unequal
      but do not differ greatly, the Hartley test may still be used
      as an approximate test (average df are used).




4775453 & P subcapitata 96-hr cell density (mg/L)
File: 0301c       Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance
---------------------------------------------------------------------------


Calculated B statistic =    6.75
Table Chi-square value =   15.09  (alpha = 0.01)
Table Chi-square value =   11.07  (alpha = 0.05)

Average df used in calculation  ==>  df (avg n - 1) =   2.00
Used for Chi-square table value ==>  df (#groups-1) =   5
---------------------------------------------------------------------------

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is
      used to calculate the B statistic (see above).

4775453 & P subcapitata 96-hr cell density (mg/L)
File: 0301c       Transform: NO TRANSFORMATION


                         ANOVA TABLE
---------------------------------------------------------------------------


SOURCE           DF          SS              MS              F
---------------------------------------------------------------------------

Between           5    19223869117.281   3844773823.457        0.684

Within (Error)   12    67492677120.000   5624389760.000
---------------------------------------------------------------------------
Total            17    86716546237.250
---------------------------------------------------------------------------

  Critical F value =   3.11  (0.05,5,12)
  Since  F < Critical F  FAIL TO REJECT  Ho:All groups equal



4775453 & P subcapitata 96-hr cell density (mg/L)
File: 0301c       Transform: NO TRANSFORMATION

      DUNNETTS TEST   -   TABLE 1 OF 2          Ho:Control<Treatment
---------------------------------------------------------------------------
                         TRANSFORMED   MEAN CALCULATED IN
GROUP    IDENTIFICATION     MEAN         ORIGINAL UNITS    T STAT  SIG
-----    --------------  -----------   ------------------  ------  ---
  1         Neg control 2000102.000      2000102.000
  2                 3.8 2041542.333      2041542.333       -0.677
  3                 6.4 2035928.333      2035928.333       -0.585
```

Data Evaluation Report on the Acute Toxicity of 4775453 (M07; Metabolite of BAS 800 H) to Algae (*Pseudokirchneriella subcapitata*)

PMRA Submission Number: 2008-0431
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```
    4                       11 2082289.333        2082289.333        -1.342
    5                       18 2086625.333        2086625.333        -1.413
    6                       29 2010304.333        2010304.333        -0.167
--------------------------------------------------------------------------------
Dunnett table value =  2.50      (1 Tailed Value, P=0.05,  df=12,5)
```

4775453 & P subcapitata 96-hr cell density (mg/L)
File: 0301c   Transform: NO TRANSFORMATION

          DUNNETTS TEST   -   TABLE 2 OF 2   Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	3			
2	3.8	3	153084.804	7.7	-41440.333
3	6.4	3	153084.804	7.7	-35826.333
4	11	3	153084.804	7.7	-82187.333
5	18	3	153084.804	7.7	-86523.333
6	29	3	153084.804	7.7	-10202.333

4775453 & P subcapitata 96-hr cell density (mg/L)
File: 0301c   Transform: NO TRANSFORMATION

          WILLIAMS TEST   (Isotonic regression model)   TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	3	2000102.000	2000102.000	2000102.000
2	3.8	3	2041542.333	2041542.333	2038735.333
3	6.4	3	2035928.333	2035928.333	2038735.333
4	11	3	2082289.333	2082289.333	2059739.667
5	18	3	2086625.333	2086625.333	2059739.667
6	29	3	2010304.333	2010304.333	2059739.667

4775453 & P subcapitata 96-hr cell density (mg/L)
File: 0301c   Transform: NO TRANSFORMATION

          WILLIAMS TEST   (Isotonic regression model)   TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Neg control	2000102.000				
3.8	2038735.333	0.631		1.78	k= 1, v=12
6.4	2038735.333	0.631		1.87	k= 2, v=12
11	2059739.667	0.974		1.90	k= 3, v=12

Data Evaluation Report on the Acute Toxicity of 4775453 (M07; Metabolite of BAS 800 H) to Algae (*Pseudokirchneriella subcapitata*)

PMRA Submission Number: 2008-0431
PMRA Document ID: 1664711   EPA MRID Number: 47560301

```
                   182059739.667     0.974              1.92      k= 4, v=12
                   292059739.667     0.974              1.93      k= 5, v=12
----------------------------------------------------------------------------
s = 74995.932
Note: df used for table values are approximate when v > 20.

 0301C : 4775453 & P subcapitata 96-hr cell density (mg/L)
----------------------------------------------------------------------------
Williams Test
----------------------------------------------------------------------------
[One-Sided Test for Decrease, alpha =     0.050000 ]

Dose          Isotone     T-bar P-value  Significance
              Means
----------------------------------------------------------
0             2.05E+06          .
3.8           2.05E+06     -0.8034    N.S.
6.4           2.05E+06     -0.8034    N.S.
11            2.05E+06     -0.8034    N.S.
18            2.05E+06     -0.8034    N.S.
29            2.01E+06     -0.1666    N.S.

 "*"=Significant; "N.S."=Not Significant.

!!!Failure #3:  Data not suitable for probit model fit.

Criterion is 3 or more distinct isotone means.


4775453 & P subcapitata 96-hour biomass (mg/L)
File: 0301b       Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies
----------------------------------------------------------------------------

INTERVAL      <-1.5      -1.5 to <-0.5     -0.5 to 0.5    >0.5 to 1.5     >1.5
              _____      _____     _____    _____     ____

EXPECTED      1.206      4.356             6.876          4.356           1.206
OBSERVED      0          6                 5              7               0


----------------------------------------------------------------------------
Calculated Chi-Square goodness of fit test statistic =   5.1491
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.



4775453 & P subcapitata 96-hour biomass (mg/L)
File: 0301b       Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
----------------------------------------------------------------------------

D = 353679630066176.000
```

Data Evaluation Report on the Acute Toxicity of 4775453 (M07; Metabolite of BAS 800 H) to Algae (*Pseudokirchneriella subcapitata*)

PMRA Submission Number: 2008-0431
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```
W =    0.964

Critical W (P = 0.05) (n = 18) = 0.897
Critical W (P = 0.01) (n = 18) = 0.858
------------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.



4775453 & P subcapitata 96-hour biomass (mg/L)
File: 0301b          Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
------------------------------------------------------------------------

Calculated H statistic (max Var/min Var) =   22.27
Closest, conservative, Table H statistic = 1362.0  (alpha = 0.01)

Used for Table H ==>     R (# groups) =   6,    df (# reps-1) =      2
Actual values    ==>     R (# groups) =   6,    df (# avg reps-1) =  2.00


------------------------------------------------------------------------

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
      but do not differ greatly, the Hartley test may still be used
      as an approximate test (average df are used).



4775453 & P subcapitata 96-hour biomass (mg/L)
File: 0301b          Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance
------------------------------------------------------------------------

Calculated B statistic =    4.99
Table Chi-square value =   15.09   (alpha = 0.01)
Table Chi-square value =   11.07   (alpha = 0.05)

Average df used in calculation  ==>   df (avg n - 1) =   2.00
Used for Chi-square table value ==>   df (#groups-1) =   5
------------------------------------------------------------------------

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is
      used to calculate the B statistic (see above).


4775453 & P subcapitata 96-hour biomass (mg/L)
File: 0301b          Transform: NO TRANSFORMATION
```

Data Evaluation Report on the Acute Toxicity of 4775453 (M07; Metabolite of BAS 800 H) to Algae (*Pseudokirchneriella subcapitata*)

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	87473762539136.000	17494752507824.000	0.594
Within (Error)	12	353679629877248.000	29473302489760.000	
Total	17	441153392416256.000		

```
  Critical F value =   3.11   (0.05,5,12)
  Since  F < Critical F  FAIL TO REJECT  Ho:All groups equal
```

4775453 & P subcapitata 96-hour biomass (mg/L)
File: 0301b   Transform: NO TRANSFORMATION

DUNNETTS TEST   -   TABLE 1 OF 2   Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Neg control	82392536.000	82392536.000		
2		3.880886596.000	80886596.000	0.340	
3		6.476959892.000	76959892.000	1.226	
4		1179069208.000	79069208.000	0.750	
5		1875861872.000	75861872.000	1.473	
6		2978723244.000	78723244.000	0.828	

Dunnett table value = 2.50   (1 Tailed Value, P=0.05, df=12,5)

4775453 & P subcapitata 96-hour biomass (mg/L)
File: 0301b   Transform: NO TRANSFORMATION

DUNNETTS TEST   -   TABLE 2 OF 2   Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	3			
2	3.8	3	11081761.008	13.4	1505940.000
3	6.4	3	11081761.008	13.4	5432644.000
4	11	3	11081761.008	13.4	3323328.000
5	18	3	11081761.008	13.4	6530664.000
6	29	3	11081761.008	13.4	3669292.000

4775453 & P subcapitata 96-hour biomass (mg/L)
File: 0301b   Transform: NO TRANSFORMATION

Data Evaluation Report on the Acute Toxicity of 4775453 (M07; Metabolite of BAS 800 H) to Algae (*Pseudokirchneriella subcapitata*)

PMRA Submission Number: 2008-0431
PMRA Document ID: 1664711   EPA MRID Number: 47560301

```
           WILLIAMS TEST   (Isotonic regression model)    TABLE 1 OF 2
----------------------------------------------------------------------------
GROUP                                  ORIGINAL      TRANSFORMED     ISOTONIZED
          IDENTIFICATION       N         MEAN          MEAN            MEAN
------   -------------------  ---   -------------   -------------   -----------
   1            Neg control   382392536.000   82392536.000   82392536.000
   2                    3.8   380886596.000   80886596.000   80886596.000
   3                    6.4   376959892.000   76959892.000   78014550.000
   4                     11   379069208.000   79069208.000   78014550.000
   5                     18   375861872.000   75861872.000   77292558.000
   6                     29   378723244.000   78723244.000   77292558.000
----------------------------------------------------------------------------
```

```
4775453 & P subcapitata 96-hour biomass (mg/L)
File: 0301b        Transform: NO TRANSFORMATION

           WILLIAMS TEST  (Isotonic regression model)    TABLE 2 OF 2
----------------------------------------------------------------------------
                   ISOTONIZED    CALC.     SIG     TABLE      DEGREES OF
    IDENTIFICATION    MEAN      WILLIAMS   P=.05   WILLIAMS    FREEDOM
---------------------------------------------------------------------------
       Neg control82392536.000
              3.880886596.000    0.340             1.78       k= 1,  v=12
              6.478014550.000    0.988             1.87       k= 2,  v=12
              1178014550.000     0.988             1.90       k= 3,  v=12
              1877292558.000     1.151             1.92       k= 4,  v=12
              2977292558.000     1.151             1.93       k= 5,  v=12
----------------------------------------------------------------------------
s = 5428931.984
Note: df used for table values are approximate when v > 20.
```

```
4775453 & P subcapitata 96-hr growth rate (mg/L)
File: 0301g        Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies
------------------------------------------------------------------------------

INTERVAL     <-1.5      -1.5 to <-0.5    -0.5 to 0.5    >0.5 to 1.5    >1.5


EXPECTED     1.206         4.356           6.876          4.356        1.206
OBSERVED     0             7               4              7            0

------------------------------------------------------------------------------
Calculated Chi-Square goodness of fit test statistic =   6.8246
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.
```

Data Evaluation Report on the Acute Toxicity of 4775453 (M07; Metabolite of BAS 800 H) to Algae (*Pseudokirchneriella subcapitata*)

PMRA Submission Number: 2008-0431
PMRA Document ID: 1664711   EPA MRID Number: 47560301

```
4775453 & P subcapitata 96-hr growth rate (mg/L)
File: 0301g        Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
--------------------------------------------------------------------------------

D =  176.667

W =    0.962

Critical W (P = 0.05) (n = 18) = 0.897
Critical W (P = 0.01) (n = 18) = 0.858
--------------------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.



4775453 & P subcapitata 96-hr growth rate (mg/L)
File: 0301g        Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
--------------------------------------------------------------------------------

Calculated H statistic (max Var/min Var) =  124.00
Closest, conservative, Table H statistic = 1362.0  (alpha = 0.01)

Used for Table H ==>    R (# groups) =  6,    df (# reps-1) =      2
Actual values    ==>    R (# groups) =  6,    df (# avg reps-1) =  2.00


--------------------------------------------------------------------------------

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
      but do not differ greatly, the Hartley test may still be used
      as an approximate test (average df are used).



4775453 & P subcapitata 96-hr growth rate (mg/L)
File: 0301g        Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance
--------------------------------------------------------------------------------

Calculated B statistic =    7.17
Table Chi-square value =   15.09  (alpha = 0.01)
Table Chi-square value =   11.07  (alpha = 0.05)

Average df used in calculation  ==>   df (avg n - 1) =   2.00
Used for Chi-square table value ==>   df (#groups-1) =   5
--------------------------------------------------------------------------------

Data PASS homogeneity test at 0.01 level. Continue analysis.
```

Data Evaluation Report on the Acute Toxicity of 4775453 (M07; Metabolite of BAS 800 H) to Algae (*Pseudokirchneriella subcapitata*)

PMRA Submission Number: 2008-0431
PMRA Document ID: 1664711   EPA MRID Number: 47560301

NOTE: If groups have unequal replicate sizes the average replicate size is
      used to calculate the B statistic (see above).

4775453 & P subcapitata 96-hr growth rate (mg/L)
File: 0301g   Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	53.333	10.667	0.725
Within (Error)	12	176.667	14.722	
Total	17	230.000		

   Critical F value =   3.11   (0.05,5,12)
   Since F < Critical F   FAIL TO REJECT   Ho:All groups equal

4775453 & P subcapitata 96-hr growth rate (mg/L)
File: 0301g   Transform: NO TRANSFORMATION

     DUNNETTS TEST   -   TABLE 1 OF 2   Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Neg control	551.667	551.667		
2	3.8	554.000	554.000	-0.745	
3	6.4	553.667	553.667	-0.638	
4	11	556.000	556.000	-1.383	
5	18	556.333	556.333	-1.490	
6	29	552.333	552.333	-0.213	

Dunnett table value = 2.50   (1 Tailed Value, P=0.05,   df=12,5)

4775453 & P subcapitata 96-hr growth rate (mg/L)
File: 0301g   Transform: NO TRANSFORMATION

     DUNNETTS TEST   -   TABLE 2 OF 2   Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	3			
2	3.8	3	7.832	1.4	-2.333
3	6.4	3	7.832	1.4	-2.000
4	11	3	7.832	1.4	-4.333
5	18	3	7.832	1.4	-4.667

Data Evaluation Report on the Acute Toxicity of 4775453 (M07; Metabolite of BAS 800 H) to Algae (*Pseudokirchneriella subcapitata*)

PMRA Submission Number: 2008-0431
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```
   6                 29      3        7.832        1.4       -0.667
------------------------------------------------------------------------------
```

4775453 & P subcapitata 96-hr growth rate (mg/L)
File: 0301g   Transform: NO TRANSFORMATION

           WILLIAMS TEST   (Isotonic regression model)   TABLE 1 OF 2

GROUP   ORIGINAL   TRANSFORMED   ISOTONIZED
          IDENTIFICATION   N   MEAN   MEAN   MEAN
------   --------------   --- --------   -----------   ----------
   1   Neg control   3   551.667   551.667   551.667
   2   3.8   3   554.000   554.000   553.833
   3   6.4   3   553.667   553.667   553.833
   4   11   3   556.000   556.000   554.889
   5   18   3   556.333   556.333   554.889
   6   29   3   552.333   552.333   554.889

4775453 & P subcapitata 96-hr growth rate (mg/L)
File: 0301g   Transform: NO TRANSFORMATION

           WILLIAMS TEST   (Isotonic regression model)   TABLE 2 OF 2

                  ISOTONIZED   CALC.   SIG   TABLE   DEGREES OF
      IDENTIFICATION MEAN   WILLIAMS   P=.05   WILLIAMS   FREEDOM
      -------------- ------- --------   -----   --------   ----------
      Neg control   551.667
              3.8   553.833   0.692   1.78   k= 1, v=12
              6.4   553.833   0.692   1.87   k= 2, v=12
               11   554.889   1.029   1.90   k= 3, v=12
               18   554.889   1.029   1.92   k= 4, v=12
               29   554.889   1.029   1.93   k= 5, v=12
----
s =   3.837
Note: df used for table values are approximate when v > 20.

0301G : 4775453 & P subcapitata 96-hr growth rate (mg/L)

Williams Test

[One-Sided Test for Decrease, alpha =   0.050000]

Dose   Isotone   T-bar P-value Significance
              Means
----
0   554   .
3.8   554   -0.8512   N.S.
6.4   554   -0.8512   N.S.
11   554   -0.8512   N.S.
18   554   -0.8512   N.S.
```

**Data Evaluation Report on the Acute Toxicity of 4775453 (M07; Metabolite of BAS 800 H) to Algae (*Pseudokirchneriella subcapitata*)**

PMRA Submission Number: 2008-0431
PMRA Document ID: 1664711                                    EPA MRID Number: 47560301

```
29              552    -0.2128    N.S.

 "*"=Significant; "N.S."=Not Significant.

!!!Failure #3:  Data not suitable for probit model fit.

Criterion is 3 or more distinct isotone means.



4775453 & P subcapitata 96-hr yield (mg/L)
File: 0301y      Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies
-------------------------------------------------------------------

INTERVAL     <-1.5      -1.5 to <-0.5     -0.5 to 0.5    >0.5 to 1.5    >1.5
             ____       ____              ____           ____          ____

EXPECTED     1.206      4.356             6.876          4.356         1.206
OBSERVED     0          6                 6              6             0

-------------------------------------------------------------------
Calculated Chi-Square goodness of fit test statistic =    3.7645
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.



4775453 & P subcapitata 96-hr yield (mg/L)
File: 0301y      Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
-------------------------------------------------------------------

D = 67492677097.313

W =    0.962

Critical W (P = 0.05) (n = 18) = 0.897
Critical W (P = 0.01) (n = 18) = 0.858
-------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.



4775453 & P subcapitata 96-hr yield (mg/L)
File: 0301y      Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
-------------------------------------------------------------------

Calculated H statistic (max Var/min Var) =    84.05
Closest, conservative, Table H statistic = 1362.0  (alpha = 0.01)
```

Data Evaluation Report on the Acute Toxicity of 4775453 (M07; Metabolite of BAS 800 H) to Algae (*Pseudokirchneriella subcapitata*)

```
Used for Table H ==>    R (# groups) =   6,    df (# reps-1) =        2
Actual values     ==>    R (# groups) =   6,    df (# avg reps-1) =  2.00


- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
      but do not differ greatly, the Hartley test may still be used
      as an approximate test (average df are used).



4775453 & P subcapitata 96-hr yield (mg/L)
File: 0301y        Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

Calculated B statistic =    6.75
Table Chi-square value =   15.09   (alpha = 0.01)
Table Chi-square value =   11.07   (alpha = 0.05)

Average df used in calculation  ==>   df (avg n - 1) =   2.00
Used for Chi-square table value ==>   df (#groups-1) =   5
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is
      used to calculate the B statistic (see above).


 4775453 & P subcapitata 96-hr yield (mg/L)
File: 0301y        Transform: NO TRANSFORMATION

                          ANOVA TABLE
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

SOURCE              DF            SS                  MS              F
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

Between             5      19223869085.281    3844773817.055         0.684

Within (Error)      12     67492677120.000    5624389760.000
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
Total               17     86716546205.250
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

  Critical F value =   3.11   (0.05,5,12)
  Since  F < Critical F   FAIL TO REJECT   Ho:All groups equal



4775453 & P subcapitata 96-hr yield (mg/L)
File: 0301y        Transform: NO TRANSFORMATION
```

Data Evaluation Report on the Acute Toxicity of 4775453 (M07; Metabolite of BAS 800 H) to Algae (*Pseudokirchneriella subcapitata*)

PMRA Submission Number: 2008-0431
PMRA Document ID: 1664711   EPA MRID Number: 47560301

```
        DUNNETTS TEST    -    TABLE 1 OF 2              Ho:Control<Treatment
------------------------------------------------------------------------------
                                 TRANSFORMED     MEAN CALCULATED IN
        GROUP     IDENTIFICATION      MEAN         ORIGINAL UNITS    T STAT  SIG
        -----   ----------------  -----------   -----------------   ------  ---
          1          Neg control 1990102.000       1990102.000
          2                  3.8 2031542.333       2031542.333       -0.677
          3                  6.4 2025928.333       2025928.333       -0.585
          4                  11  2072289.333       2072289.333       -1.342
          5                  18  2076625.333       2076625.333       -1.413
          6                  29  2000304.333       2000304.333       -0.167
------------------------------------------------------------------------------
Dunnett table value =  2.50      (1 Tailed Value, P=0.05,  df=12,5)
```

```
4775453 & P subcapitata 96-hr yield (mg/L)
File: 0301y       Transform: NO TRANSFORMATION


        DUNNETTS TEST    -    TABLE 2 OF 2              Ho:Control<Treatment
------------------------------------------------------------------------------
                           NUM OF   Minimum Sig Diff   % of      DIFFERENCE
        GROUP  IDENTIFICATION REPS  (IN ORIG. UNITS)  CONTROL  FROM CONTROL
        -----  -------------- ----  ----------------  -------  ------------
          1      Neg control    3
          2              3.8    3      153084.804       7.7    -41440.333
          3              6.4    3      153084.804       7.7    -35826.333
          4              11     3      153084.804       7.7    -82187.333
          5              18     3      153084.804       7.7    -86523.333
          6              29     3      153084.804       7.7    -10202.333
------------------------------------------------------------------------------
```

```
4775453 & P subcapitata 96-hr yield (mg/L)
File: 0301y       Transform: NO TRANSFORMATION


        WILLIAMS TEST   (Isotonic regression model)   TABLE 1 OF 2
------------------------------------------------------------------------------
GROUP                              ORIGINAL     TRANSFORMED    ISOTONIZED
          IDENTIFICATION      N      MEAN          MEAN          MEAN
------   --------------     ---  -----------   -----------   -----------
   1          Neg control   3  1990102.000    1990102.000    1990102.000
   2                  3.8   3  2031542.333    2031542.333    2028735.333
   3                  6.4   3  2025928.333    2025928.333    2028735.333
   4                  11    3  2072289.333    2072289.333    2049739.667
   5                  18    3  2076625.333    2076625.333    2049739.667
   6                  29    3  2000304.333    2000304.333    2049739.667
------------------------------------------------------------------------------
```

```
4775453 & P subcapitata 96-hr yield (mg/L)
File: 0301y       Transform: NO TRANSFORMATION
```

Data Evaluation Report on the Acute Toxicity of 4775453 (M07; Metabolite of BAS 800 H) to Algae (*Pseudokirchneriella subcapitata*)

PMRA Submission Number: 2008-0431
PMRA Document ID: 1664711   EPA MRID Number: 47560301

```
          WILLIAMS TEST   (Isotonic regression model)    TABLE 2 OF 2
-------------------------------------------------------------------------
                    ISOTONIZED   CALC.     SIG      TABLE     DEGREES OF
      IDENTIFICATION    MEAN     WILLIAMS  P=.05    WILLIAMS  FREEDOM
-------------------------------------------------------------------------
          Neg control1990102.000
                  3.82028735.333   0.631            1.78      k= 1, v=12
                  6.42028735.333   0.631            1.87      k= 2, v=12
                  112049739.667    0.974            1.90      k= 3, v=12
                  182049739.667    0.974            1.92      k= 4, v=12
                  292049739.667    0.974            1.93      k= 5, v=12
-------------------------------------------------------------------------
s = 74995.932
Note: df used for table values are approximate when v > 20.


  0301Y : 4775453 & P subcapitata 96-hr yield (mg/L)
-------------------------------------------------------------------------

Williams Test
-------------------------------------------------------------------------
[One-Sided Test for Decrease, alpha =     0.050000 ]

Dose        Isotone    T-bar P-value  Significance
            Means
-------------------------------------------------------------------------
0           2.04E+06          .
3.8         2.04E+06   -0.8034     N.S.
6.4         2.04E+06   -0.8034     N.S.
11          2.04E+06   -0.8034     N.S.
18          2.04E+06   -0.8034     N.S.
29             2E+06   -0.1666     N.S.

 "*"=Significant; "N.S."=Not Significant.

!!!Failure #3:  Data not suitable for probit model fit.

Criterion is 3 or more distinct isotone means.
```